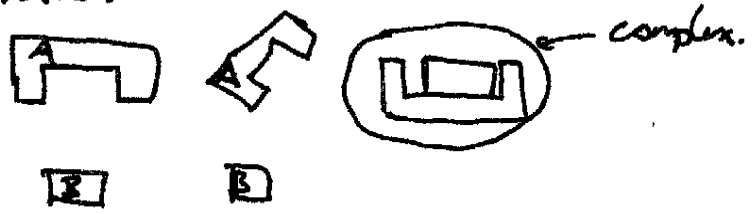
Chapter 4: Molecular Associations

4.1 Equilibrium in solution

$$A + B \rightleftharpoons C$$

protein, ligand, complex



In equilibrium the respective concentrations are

$$\frac{[C]}{[A][B]} = \frac{1}{K_{diss}}$$ ← "dissociation constant"

where did this come from? Ans. Interplay of entropy of translation and free energy difference of binding.

Recall from chapter 1. The free energy of a concentration [A] of molecules in solution

$$G = G^\circ + RT \log [A]$$ ← concentration in units of 1M.

↑ "molar free energy" ← Free energy of one mole of A in solution.

We computed the $\log [A]$ term from the translational entropy of A molecules in the box.

Now consider the free energy change when A and B make one complex C.

$$\Delta G = G_C - (G_A + G_B) = G_C^\circ - (G_A^\circ + G_B^\circ) + RT \log\left[\frac{[C]}{[A][B]}\right]$$

we assume low enough concentrations that there are no significant interactions between A and B

call this ΔG° ← the free energy change associated w/ $[M_A] + [M_B] \rightarrow [M_C]$

In equilibrium G is a minimum so $\Delta G = 0 \Rightarrow$

$$\Delta G^\circ = -RT \log\left(\frac{[C]}{[A][B]}\right) \Rightarrow \frac{[C]}{[A][B]} = e^{-\Delta G^\circ/RT}$$

so $K_{diss} = \exp\{\Delta G^\circ/RT\}$

Now let's look at the fraction f the fraction of bound protein as a function of the concentration of the ligand.

$[P] = [P_f] + [P_b]$

↑ protein concentration ↑ free ↑ bound.

Changing the letters in our first ~~exp~~ example we get

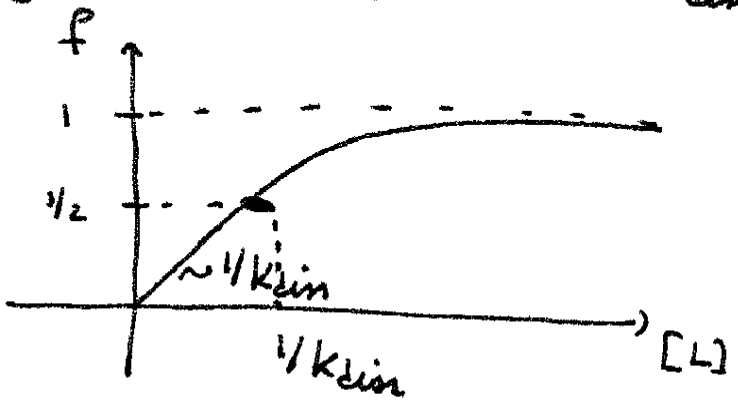
bound protein → $\dfrac{[P_b]}{[P]_f [L]} = \dfrac{[P_b]}{([P]-[P_b])[L]} = \dfrac{1}{K_{diss}}$

free protein ↗ ↑ Ligand

$\left(\dfrac{f}{1-f}\right) = \dfrac{[L]}{K_{diss}}$. Solve for $f = \dfrac{[P_b]}{[P]}$

$f = (1-f)\alpha \Rightarrow (1+\alpha)f = \alpha$; $\alpha = [L]/K_{diss}$

$$f = \frac{[L]}{[L] + K_{diss}}$$



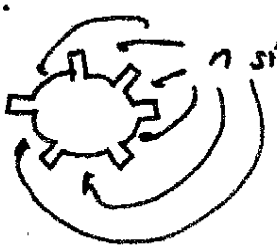
How can we tune this reaction to be sharper? We (and biology) might want to use protein-ligand association as a signalling mechanism. In that case we might require a sharper on/off switch!

Ans: Our old friend cooperativity.

4.2 Concerted Binding and Perfect Cooperativity.

all or nothing w/ two binding sites.

We can imagine n binding sites as well.



$P_0 + nL \rightleftharpoons P_n$ ← full protein.

↑ empty protein

↖ n copies of the ligand

Now, how does the fraction of bound protein depend on [L]?

Consider the statistical mechanics of this reaction in a box of volume V.

Partition sum of N_0 free protein
N_L ligands
N_b bound protein.

$f = \frac{N_b}{N_0}$ Now the number of ligands in solution is

$n_L = N_L - n f N_0$ since each of N_b bound protein eats up n ligands.

Recall the partition sum of particles in a box.

$$Z_L = \frac{1}{(n_L)!}\left(e^{-\bar{G}/k_BT}\right)^{n_L}\left(\frac{V}{\lambda^3}\right)^{n_L} \leftarrow \text{for free ligands.}$$

↑ free ligands. ↑ internal degrees of freedom ↖ translational degrees of freedom.

Do the same for all the other chemical species:

$$Z = Z_L Z_b Z_0 = \frac{1}{(n_L)!}\frac{1}{(N_b)!}\frac{1}{(N_0-N_b)!} e^{-\left(\bar{G}_L n_L + \bar{G}_B N_b + \bar{G}_0 \cdot (N_0-N_b)\right)/k_BT} \left(\frac{V}{\lambda^3}\right)^{n_L}\left(\frac{V}{\lambda^3}\right)^{N_b}\left(\frac{V}{\lambda^3}\right)^{N_0-N_b}$$

To get the equilibrium fraction of bound protein, let's minimize $G = -k_BT \log Z$ wrt f.

First of all:

$\exp\{-(\bar{G}_L n_L + \bar{G}_B N_b + \bar{G}_0(N_0 - N_b))/k_BT\}$ can be simplified:

$$\{-\bar{G}_L(N_L - nfN_0) - \bar{G}_B f N_0 - \bar{G}_0 N_0 + \bar{G}_0 f N_0\}/k_BT$$

$$= \{-\bar{G}_L(N_L - nfN_0) - (\bar{G}_B - \bar{G}_0) f N_0 - \bar{G}_0 N_0\}/k_BT$$

↑ call this $\Delta\bar{G}$ = change in free energy of the protein between "loaded" and "empty" states.

Now recall $\log N! \cong N\log N - N$

so $\log\left(\frac{1}{n_L!}\frac{1}{N_b!}\frac{1}{(N_0-N_b)!}\right) = -n_L\log(n_L) + n_L +$

$- N_b \log N_b + N_b - (N_0 - N_b)\log(N_0 - N_b) + N_0 - N_b$

(cancel)

$$= -(N_L - nfN_0)\log(N_L - nfN_0) + (N_L - nfN_0) - fN_0\log(fN_0)$$
$$- N_0(1-f)\log[N_0(1-f)] + N_0$$

$$= -(N_L - nfN_0)\log(N_L - nfN_0) + (N_L - nfN_0) - fN_0\log(fN_0) +$$
$$- N_0(1-f)\log[(1-f)N_0] + N_0$$

Now we can put everything together. Keep in mind that $\bar{V}$, N_0, and N_L are fixed. These are the volume of the box and the number of molecules in it.

The chemical reaction can't change these, but can change f.

$$G = + \bar{G}_L(N_L - nfN_0) + \Delta\bar{G} f N_0 - k_B T \log\left(\left(\frac{V}{\lambda^3}\right)^{n_L}\left(\frac{V}{\lambda^3}\right)^{N_0}\right) + k_B T \{$$

$$(N_L - nfN_0)\log(N_L - nfN_0) - (N_L - nfN_0) + fN_0 \log(fN_0) +$$

$$N_0(1-f)\log[(1-f)N_0] - N_0\}$$

and $n_L = N_L - nfN_0$

~~$\frac{\partial G}{\partial f} = 0 \Rightarrow G = -nN_0\bar{G}_L + \Delta G N_0$~~ Let's simplify this a bit more...

$$G = \bar{G}_L n_L + \Delta\bar{G} f N_0 - k_B T n_L \log(V/\lambda^3) - N_0 k_B T \log(V/\lambda^3)$$

$$+ k_B T\{n_L \log(n_L) - n_L + fN_0 \log(f) + fN_0 \log N_0 + N_0(1-f)\log N_0$$

$$+ N_0(1-f)\log(1-f) - N_0\}$$

(canal.)

$$G = \bar{G}_L n_L + \Delta\bar{G} f N_0 + k_B T n_L \log\left(\frac{\lambda^3 n_L}{Ve}\right) + k_B T N_0 \log\left(\frac{\lambda^3 N_0}{Ve}\right)$$

$$+ k_B T\{fN_0 \log f + N_0(1-f)\log(1-f)\}$$

Now $\frac{\partial G}{\partial f} = 0 \Rightarrow$

$$0 = \Delta\bar{G} N_0 + k_B T(-nN_0)\log\left(\frac{\lambda^3 n_L}{Ve}\right) + k_B T n_L \frac{Ve}{\lambda^3 n_L}\frac{\lambda^3}{Ve}(-nN_0)$$

$$+ k_B T\{N_0 \log f + N_0 \cdot 1 + (-1) - \log(1-f)\}$$

$$0 = \Delta\bar{G} N_0 - n k_B T N_0 \left[\log\left(\frac{\lambda^3 n_L}{\nu e}\right) + 1\right] + N_0 k_B T \left\{\log\left(\frac{f}{1-f}\right)\right\}$$

$$\frac{\Delta\bar{G}}{k_B T} = n \log\left(\frac{\lambda^3}{\nu}(N_L - n f N_0)\right) + \log\left(\frac{f}{1-f}\right)$$

↑ concentration of free ligand.

$$+ \log(K_{diss})$$

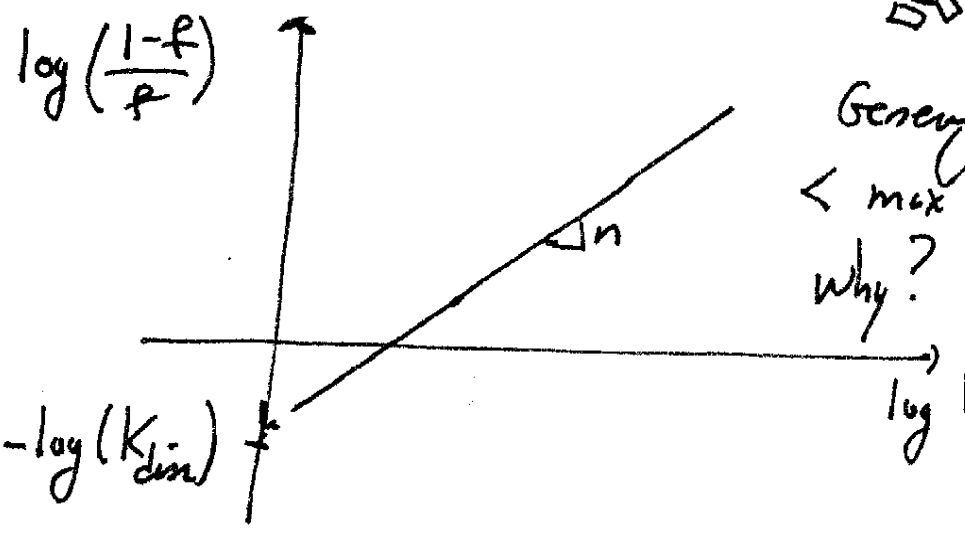
$$\Rightarrow \quad +\log(K_{diss}) = n \log([L]) - \log\left(\frac{1-f}{f}\right)$$

$$\text{so} \quad \log\left(\frac{1-f}{f}\right) = n \log[L] - \log(K_{diss})$$

$$\frac{1-f}{f} = [L]^n / K_{diss} \Rightarrow \boxed{f = \frac{[L]^n}{[L]^n + K_{diss}}}$$

layer $n \Rightarrow$ sharper transitions.

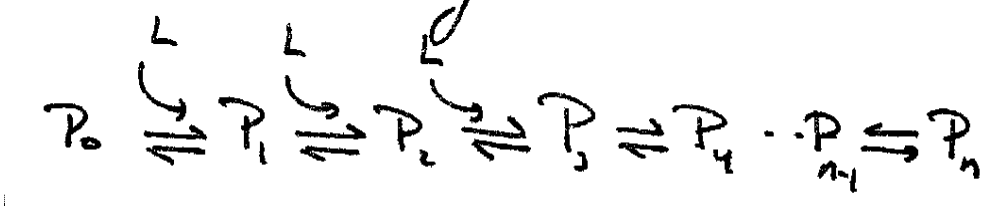
Hill Plot.



Generally the slope is < max # binding sites. Why? Ans. Partial cooperativity!

4.2.2 Sequential Binding.

empty state — Partially loaded states — loaded state.



We now have multiple reactions going on. For each one we can write.

$$\frac{[P_j]}{[L][P_{j-1}]} = \frac{1}{K_j}.$$

what is f now? We should say that $[P_j]$ contributes $\frac{j}{n}$ to f since at fully loaded each protein holds n ligands.

$$f = \frac{[P_1] + 2[P_2] + \cdots + n[P_n]}{n} \times \frac{1}{[P_{tot}]}$$

$$\boxed{f = \frac{1}{n} \sum_{k=1}^{n} k[P_k] \frac{1}{[P_{tot}]}}$$

But how to compute $[P_k]$ in chemical equilibrium?

Note $[P_n] = \frac{1}{K_n}[L][P_{n-1}]$; $[P_{n-1}] = \frac{1}{K_{n-1}}[L][P_{n-2}]$

and so on.

For three steps: $[P_3] = \frac{1}{K_3}[L][P_2]$

$$[P_2] = \frac{1}{K_2}[L][P_1]$$

$$[P_1] = \frac{1}{K_1}[L][P_0]$$

$$\Rightarrow [P_3] = \frac{1}{K_3 K_2 K_1}[L]^3[P_0]; \quad [P_2] = \frac{1}{K_2 K_1}[L]^2[P_0]$$

In general: $[P_k] = \frac{1}{K_k \cdots K_1}[L]^k[P_0]$

Now we can compute f:

$$f = \frac{1}{n}\frac{1}{[P_{tot}]}\left(\sum_{k=1}^{n} k\frac{[L]^k}{\prod_{j=1}^{k} K_j}\right)[P_0]$$

what is $[P_{tot}]$? Well, all proteins have 0 to n ligands so:

$$[P_{tot}] = \sum_{k=0}^{n}\frac{[L]^k}{\prod_{j=1}^{k} K_j}[P_0]$$

Let's write the answer for $n = 2 \Rightarrow P_0, P_1, P_2$ are all of the possible states.

$$f = \frac{1}{2}\frac{1}{\left(1 + \frac{[L]}{K_1} + \frac{[L]^2}{K_2 K_1}\right)}\left(\frac{[L]}{K_1} + \frac{2[L]^2}{K_2 K_1}\right)$$

P_0 P_1 P_2

Let's compare our new result with our answer

for perfect cooperativity.

$$f_{pc} = \frac{[L]^2}{[L]^2 + K}$$

↑ perfect cooperativity.

Our new result is less steep unless we take $K_1 \to \infty$ with $K_1 K_2^2 K$ finite

Then $f \to \frac{1}{2} \frac{1}{(1 + [L]/k)} \frac{2[L]^2}{K} = \frac{[L]^2}{[L]^2 + K}$

What does this mean? We forced ($K_1 \to \infty$) the singly loaded protein to drop its ligand or get another one. That's pc. And pc gives the sharpest on/off switch possible.

Note we have said nothing about reaction rates - that is for chapter 10.

Chapter 5: Allosteric Interactions

The allosteric transition $T \rightleftharpoons R$

↑ tense ↑ relaxed.

$T_0 + L \rightleftharpoons T_1$ binds poorly

$R_0 + L \rightleftharpoons R_1$ binds strongly.

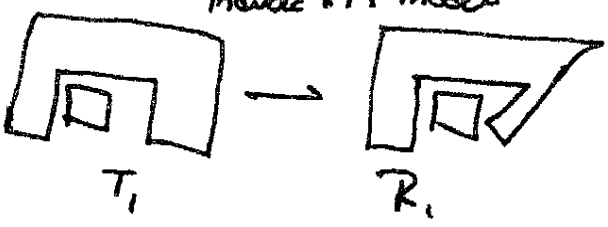
We have a chemical equilibrium of 4 forms.

$$T_0 + L \overset{K_T}{\rightleftharpoons} T_1$$

$$Y_0 \uparrow\downarrow \qquad \uparrow\downarrow Y_1$$

$$R_0 + L \overset{K_R}{\rightleftharpoons} R_1$$

"induced fit model"



A = Total fraction of the protein in the R state.

$$A = \frac{[R_0] + [R_1]}{[R_0] + [R_1] + [T_0] + [T_1]}$$

The equilibrium constants of the various reactions are.

$$K_T = \frac{[T_1]}{[T_0][L]}; \quad K_R = \frac{[R_1]}{[R_0][L]}; \quad Y_0 = \frac{[R_0]}{[T_0]}; \quad Y_1 = \frac{[R_1]}{[T_1]}$$

After a bit of algebra

$$A = \frac{[R_0] + [L] K_R [R_0]}{[R_0] + [L] K_R [R_0] + [R_0]/Y_0 + [L] K_R [R_0]/Y_1}$$

cancelling the common factor of $[R_0]$.

$$A = \frac{1 + [L] K_R}{1 + Y_0^{-1} + [L] K_R (1 + Y_1^{-1})}$$

so A goes from $\frac{1}{1+Y_0^{-1}}$ at $[L] = 0$ to

$$\frac{1}{1 + Y_1^{-1}} \text{ as } [L] \to \infty.$$

as you might have guessed. Why?

Note if $Y_1 \gg 1$ and $Y_0 \ll 1$

$$A \approx \frac{[L] K_R Y_0}{1 + [L] K_R Y_0}$$ ← just an effective binding constant $K_R Y_0$. ← "apparent affinity"

5.3 Binding and Response.

Note that the apparent affinity is all you can measure.
Look at the fraction of occupied sites.

$$\bar{B} = \frac{[R_1] + [T_1]}{[R_0] + [R_1] + [T_0] + [T_1]}$$ ← # occupied binding sites. ← Total # binding sites.

once again $\bar{B} \sim \frac{[L] K_R Y_0}{1 + [L] K_R Y_0}$

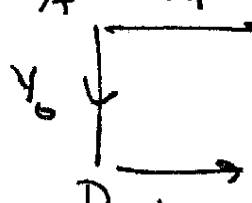
$$\Rightarrow K_{app} = K_R Y_0$$

↑ apparent affinity. in $P_0 + L \rightleftharpoons P_1$

5.4 Energy balance.

Recall $K_{diss} = e^{\Delta G^\circ / RT} = \frac{[A][B]}{[C]}$ in $A + B \rightleftharpoons C$.

and $\Delta G^\circ_{A \to B \to C} = \Delta G^\circ_{A \to D \to C}$ ← This is a state function!



$$\Rightarrow \Delta G^\circ_{A \to B} + \Delta G^\circ_{B \to C} = \Delta G^\circ_{A \to D} + \Delta G^\circ_{D \to C}$$

or $RT\{\log K_T + \log Y_1\} = RT\{\log Y_0 + \log K_R\}$

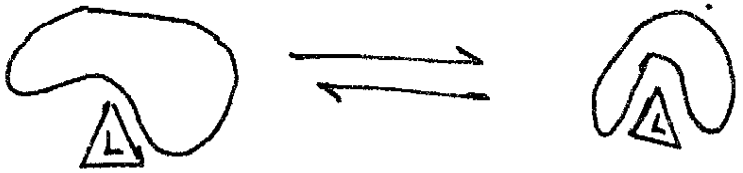
Thermodynamic Requirement. $\Rightarrow$ $\boxed{K_T Y_1 = K_R Y_0}$ ← Both are K_{app}.

conformational change.

improvement of ligand binding

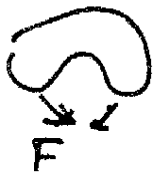
or $\frac{Y_1}{Y_0} = \frac{K_R}{K_T}$ ligand binding change factor equal to the factor by which $Y_0 \to Y_1$.

How can we think about this?



T R

improvement of protein ligand fit by doing work on the structure of the protein



work of deformation.

Binding Site interactions and the MWC Model.

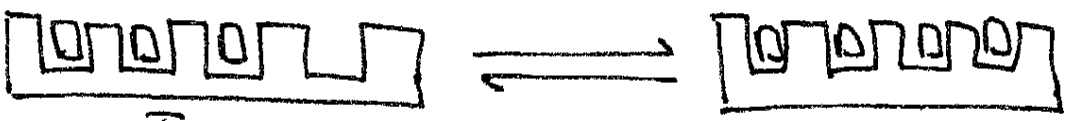
K_T and K_R describing binding to individual sites.

n sites for binding.

$$\begin{array}{ccccccc} T_0 + L & \overset{n K_T}{\rightleftharpoons} & T_1 + L & \overset{\frac{1}{2}(n-1) K_T}{\rightleftharpoons} & T_2 + L & \cdots \\ Y_0 \downarrow\uparrow & & \uparrow\downarrow Y_1 & & \uparrow\downarrow Y_2 & \\ R_0 + L & \underset{n K_R}{\rightleftharpoons} & R_1 + L & \underset{\frac{1}{2}(n-1) K_R}{\rightleftharpoons} & R_2 & \cdots \end{array}$$

Before we had $K_T = \frac{[T_1]}{[T_0][L]} \xrightarrow{\text{now}} K_T = \frac{(i+1)[T_{i+1}]}{(n-i)[T_i][L]}$

why?



and K_T, this dissociation constant, given

$$K_T = \frac{[\text{complex}]}{[A][B]} \quad A+B$$

for this case $K_T = \frac{4[T_4]}{(4-3)[T_3][L]}$ ← 4 ways to take one ligand off.

↖ 4−3 ways to fill the empty spot.

So in general $K_T = \frac{(i+1)[T_{i+1}]}{(n-i)[T_i][L]}$ and

equivalently $K_R = \frac{(i+1)[R_{i+1}]}{(n-i)[R_i][L]}$ for the R state.

As before $Y_i = \frac{[R_i]}{[T_i]}$

What is the fraction of occupied binding sites in equilibrium with ligand concentration $[L]$?

$$\bar{B} = \frac{\left(\sum_{i=0}^{n} i[R_i] + \sum_{i=0}^{n} i[T_i]\right)}{n\sum_{i=0}^{n}([R_i]+[T_i])}$$

$\bar{B}$ ↑ binding site fraction. — occupied binding sites. — Total binding sites

Now we can do the same recursion as before:

$$[T_{i+1}] = \frac{K_T(n-i)}{(i+1)}[L][T_i]$$

so $[T_1] = K_T[L]\left(\frac{n-0}{1+0}\right)[T_0]$

$[T_2] = K_T[L]\frac{(n-1)}{(1+1)}[T_1]$ and so on...

We can guess the general term

$$[T_i] = (K_T[L])^i \frac{n(n-1)(n-2)\cdots(n-i+1)}{1\cdot 2\cdot 3\cdots i}[T_0]$$

$$= (K_T[L])^i \frac{n!}{(n-i)!\,i!}[T_0]$$

To check this, plug into our recursion relation

$$[T_{i+1}] = \frac{K_T(n-i)[L]}{(i+1)}[T_i]$$

$$= (K_T[L])\frac{n-i}{(i+1)}\left\{(K_T[L])^i \frac{n!}{(n-i)!\,i!}\right\}[T_0]$$

$$= \frac{(K_T[L])^{i+1}}{(i+1)!}\frac{n!\,(n-i)}{(n-i)!} = \frac{(K_T[L])^{i+1}}{(i+1)!}\frac{n!}{(n-i-1)!}[T_0]$$

all i's have been replaced by (i+1).

Similarly $[R_i] = \frac{n!}{(n-i)!\,i!}[R_0](K_R[L])^i$

Now the sums is Bare easy since this is just the binomial expansion!

Recall: $(p+q)^n = \sum_{i=0}^{n} \frac{n!}{i!\,(n-i)!} p^{n-i} q^i$

$\binom{n}{i}$ = "n choose i" The way you read this.

So

$$\sum_{i=0}^{\infty}[T_i] = \sum_{i=0}^{\infty}\frac{n!}{(n-i)!\,i!}[T_0](K_T[L])^i = [T_0](1+K_T[L])^n$$

↑ all P=1 and q

Now, how do we do the sum

$$\sum_{i=0}^{n} i \frac{n!}{(n-i)!\, i!} (K_T[L])^i \; ?$$

Go back to $(p+q)^n = \sum_{i=0}^{n} \frac{n!}{i!(n-i)!} p^{n-i} q^i$ the Binomial Expansion

Note $q \frac{\partial}{\partial q}(p+q)^n = \sum_{i=0}^{n} \frac{n!}{i!(n-i)!} i\, p^{n-i} q^i$

so take $q \frac{\partial}{\partial q}(p+q)^n$ and then set $p=1$

$q = K_T[L]$

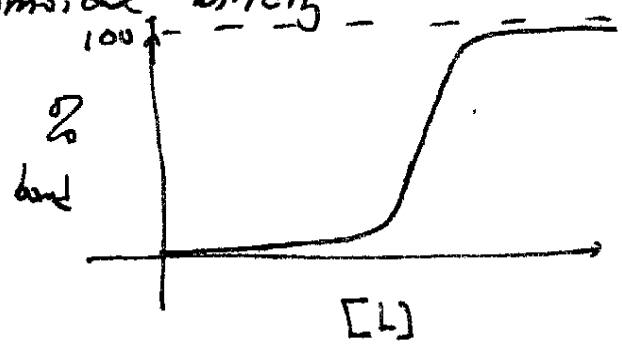
$$\Rightarrow \quad q\, n (p+q)^{n-1} \longrightarrow q n (1+q)^{n-1}$$

or $$\sum_{i=0}^{n} i [T_i] = [T_0]\, n\, K_T[L] \left(1 + K_T[L]\right)^{n-1}$$

Plug these into B and recall $[T_0] = [R_0]/\gamma_0$.

$$\Rightarrow \quad B = \frac{K_R[L]\left(1+K_R[L]\right)^{n-1} + K_T[L]\gamma_0^{-1}\left(1+K_T[L]\right)^{n-1}}{\left(1+K_R[L]\right)^n + \gamma_0^{-1}\left(1+K_T[L]\right)^n}$$

sigmoidal binding



How is this different from the Hill equation with concerted binding?

Recall $B = \underset{\text{hill}}{\dfrac{[L]^n}{[L]^n + K_{diss}}}$

Here the cooperativity arises in the fact that all sites are either in the T or R state simultaneously.

all R or all T

Not cooperative binding but a cooperativity through a global transition for the protein.

Note unlike in the Hill equation, we are forced to take $n \in \mathbb{Z}$. e.g. Hemoglobin Hill $n \cong 2.7$

But now we take $n = 4$

A few notes:
* Hemoglobin well fit by MWC model
* Binding is really noncooperative if the protein is fixed in the R and T state

high affinity ↑ ↖ low affinity.

* T state is actually under tension! If you break up the subunits, the protein part relax to the high affinity R state w/o oxygen.

5.9 Energetics of the MWC model

When the allosteric transition takes place the binding ΔG changes. We know how to write this in

in terms of the two dissociation constants K_T and K_R.

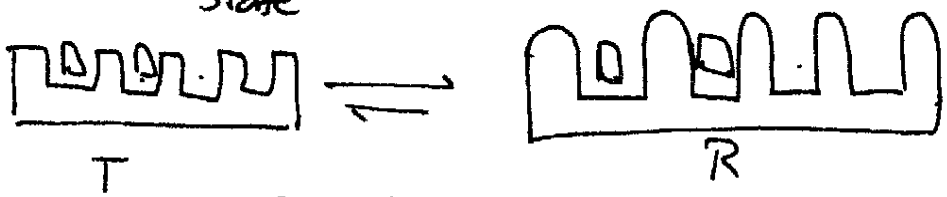
$\Delta G^\circ = RT \log(K_{diss})$ so

$$\Delta G^\circ_{binding} = -RT \log(K)$$

Now $\Delta G^{\circ R}_{binding} - \Delta G^{\circ T}_{binding} = \Delta\Delta G^\circ_{binding} = -RT \log(K_R/K_T)$

↑ Relaxed State

↑ Tense State



ΔG° of conformation transition $\Delta G^\circ_c = -RT \log Y_0$

↑ for conformational change.

Free energy change for allosteric change

$$\Delta G^\circ_a(i) = -RT \log Y_i = -RT \log Y_0 - iRT \log(K_R/K_T)$$

↑ # bound ligands.

↑ for conformation

↑ change in binding energy to the ligand.

$$\Rightarrow \frac{Y_i}{Y_0} = \left(\frac{K_R}{K_T}\right)^i$$ ← Note the factor of i

5.13 Subunit-subunit interactions: Koshland Nemethy Filmer model

MWC model ⇒ all subunits undergo T→R allosteric change in concert.

KNF model ⇒ each subunit can make its own transition.

There are now three energy scales:

① binding energy: ligand to subunit

② conformational change energy: The work required to deform a subunit from one conformation to another.

③ subunit subunit interaction energy.

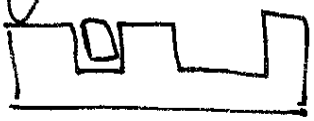
We will lump the first two together: these happen together.

⇒ free energy per binding event = free energy difference per subunit between free and occupied site

$-RT \log K_s$; K_s = binding constant that includes conformational change.

binding event

free energy cost of loaded next to empty sites.



$-RT \log \sigma$

σ is like our domain wall energy in the helix coil model.

Equilibrium between one site loaded and no sites loaded is then

$$\frac{[P_1]}{[P_0][L]} = e^{-\Delta G/RT} \quad ; \quad \Delta G = -RT\log K_s - RT\log\sigma^2 + RT\log 3$$

two "domain walls"

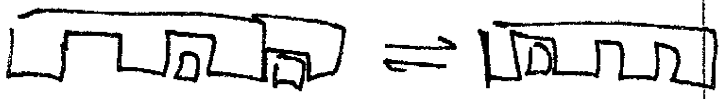
entropic factor = # ways to load the empty protein.

periodic b.c. ↝

Three site model.

$$\frac{[P_1]}{[P_0][L]} = 3K_s\sigma^2$$

Now from $P_1 \leftrightarrow P_2$:



$$\frac{[P_2]}{[P_1][L]} = e^{-\Delta G/RT} \quad ; \quad \Delta G = -RT\log K_s + 0 + RT\log 2 - RT\log 3$$

still two domain walls.

one extra bind

same entropy!

$$\Rightarrow \quad \frac{[P_2]}{[P_1][L]} = K_s$$

Finally, consider the two to three case:

$$\frac{[P_3]}{[P_2][L]} \Rightarrow^{\Delta G=} -RT\log K_s + 2RT\log\sigma - RT\log 1 + RT\log 3$$

lose two domain walls.

lose entropy

$$\Rightarrow \quad \frac{[P_3]}{[P_2][L]} = \frac{K_s}{3\sigma^2}$$

Now calculate the fraction of occupied sites as a function of [L]. The interesting point will be to see how the (σ) cooperativity parameter modifies

things.

$$\bar{B} = \frac{[P_1] + 2[P_2] + 3[P_3]}{3\{[P_0] + [P_1] + [P_2] + [P_3]\}}$$

$$[P_1] = 3K_s\sigma^2[L][P_0]$$

$$[P_2] = 3(K_s[L])^2\sigma^2[P_0]$$

$$[P_3] = \frac{K_s}{3\sigma^2}[L]\,3(K_s[L])^2\sigma^2[P_0] = \tfrac{3}{3}K_s^3[L]^3[P_0]$$

$\Rightarrow$

[redacted]

$$\bar{B} = \frac{\{3\sigma^2(K_s[L]) + 6(K_s[L])^2\sigma^2 + 3(K_s[L])^3\}[P_0]}{3\{1 + 3K_s[L]\sigma^2 + 3(K_s[L])^2\sigma^2 + [L]^3K_s^3\}[P_0]}$$

$$\bar{B} = \frac{\sigma^2(z + 2z^2) + z^3}{1 + 3\sigma^2(z + z^2) + z^3} \quad \text{where} \quad [L]K_s = z$$

Now, what does σ do?

log σ is proportional to the interaction energy

so $\sigma = 1 \Rightarrow$ no interaction.

~~$\bar{B}$ $\sigma=0$ z^3 $1+z^3$ Still Equation for constant~~

$$\bar{B} = \frac{z(1 + 2z + z^2)}{1 + 3z + 3z^2 + z^3} = \frac{z(1+z)^2}{(1+z)^3} = \frac{z}{1+z}$$

$$\Rightarrow \bar{B}_{\sigma=1} = \frac{[L]K_s}{1 + [L]K_s}$$

just the old binding curve for independent binding sites. Sands right!

Now if $\sigma \to 0$ $\log \sigma \to -\infty$ and we get a large cooperativity event that makes the intermediate loading states P_1, P_2 highly unfavorable. We get

$$\underset{\sigma \to 0}{B} = \frac{z^3}{1+z^3}$$ ← Just the Hill Equation w/ $n=3$. Afterall this is the old concerted binding theory.

What if we take $\sigma > 1$ ⟹ Anticooperative binding!

if $\sigma \gg 1$

$$B \to N\frac{1}{3}\ \frac{z(1+2z)}{z(1+z)} = \frac{1+2z}{3(1+z)}$$

and B runs from $1/3$ to $2/3$ Why? Ans. Now "domain walls" have large negative energies so the states P_0 and P_3 are highly unfavored.